Research Note

UV Inactivation of Bacteria in Apple Cider†

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ABSTRACT

Apple cider, inoculated with *Escherichia coli* and *Listeria innocua,* was processed using a simple UV apparatus. The apparatus consisted of a low-pressure mercury lamp surrounded by a coil of UV transparent tubing. Cider was pumped through the tubing at flow rates of 27 to 83 ml/min. The population of *E. coli* K-12 was reduced by 3.4 ± 0.3 log after being exposed for 19 s at a treatment temperature of 25°C. The population of *L. innocua,* which was more resistant to UV, was reduced by 2.5 ± 0.1 log after being exposed for 58 s. The electrical energy for the process was 34 J/ml and is similar to that for conventional thermal processing. UV processing has the potential to improve the safety and extend the shelf life of apple cider.

In an ideal world, all juices could be consumed without any fears about their safety. However, in the imperfect world in which we live, the safety of juices is a real concern. Therefore, most juices are pasteurized using heat. This can negatively affect their organoleptic and nutritional properties. The FDA (U.S. Food and Drug Administration) recently amended the food additive regulations to provide for the safe use of UV irradiation to reduce human pathogens and other microorganisms in juice products *(26)*. This action was in response to a food additive petition filed by California Day-Fresh Foods, Inc.

UV irradiation affects the DNA structure of harmful bacteria to render them incapable of reproduction. UV has a limited penetration depth in juices; therefore, it is necessary to apply UV to a thin film of juice. Harrington and Hills, at the Eastern Regional Research Center of the Agriculture Research Service, pioneered the use of UV to reduce the microbial population of apple cider *(13)*. Their apparatus consisted of a germicidal lamp tube that was surrounded by a quartz sleeve with an outside diameter of 2.5 cm. These were inserted into a steel outer case with an inside diameter of 3.5 cm. Cider flowed in the annulus between the quart sleeve and the steel case and formed a 1.0-cm thin film. A steel ribbon spiraled around the quartz sleeve to increase surface renewal for UV exposure. The total viable microbial count was reduced 99% by 40 s of nonthermal UV irradiation. The microbial inactivation was affected by the clarity of the cider and the length of UV exposure. UV treatment prolonged the storage life of apple cider without affecting the flavor. This type of UV apparatus has been extensively tested *(4, 8, 12, 17, 18, 20, 24, 25, 27, 28)* and is commercially available from OESCO Inc. However, it appears that few apple cider producers use this technology.

California Day-Fresh Foods, the company that petitioned the FDA to approve UV irradiation to reduce microorganisms in juice products, together with Salcor Inc., developed a UV apparatus that pumps juice through a coiled Teflon tube that is surrounded by germicidal UV lamps *(1, 2, 19)*. Following FDA approval, California Day-Fresh Foods marketed four UV-treated vegetable juices: carrot, organic carrot, and two vegetable blends *(14)*. To our knowledge, this type of apparatus is no longer being used to UV process juices.

In summary, although both the annular and tubular UV models have been extensively tested and shown to be effective in reducing microorganisms in juices, the commercial application is limited. The reasons for this are unclear; however, cost and complexity may play a part. As such, a simple UV apparatus was designed and assembled. The apparatus was similar, with one exception, to that developed by California Day-Fresh Foods. Instead of having a coil of tubing surrounded by UV lamps, the apparatus developed in this study had a UV lamp surrounded by a coil of tubing. These components are inexpensive and could easily be put together by cider producers. The objective of this study was to test the ability of the UV apparatus to inactivate gram-positive and gram-negative bacteria in apple cider.

MATERIALS AND METHODS

A gram-negative bacteria, *Escherichia coli* K-12 substrain C600 *(9)*, and a gram-positive bacteria, *Listeria innocua* SA3-VT *(5, 6)*, were supplied by P. M. Fratamico and L. K. Bagi, respectively, both of the U.S. Department of Agriculture, Wyndmoor,

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Pa. They were maintained on tryptose agar (Difco, Becton Dickinson, Sparks, Md.) at 4°C. The bacteria were cultured in brain heart infusion (Difco, Becton Dickinson) for 24 h at 37°C. The stationary-phase cells were dispensed into vials containing 20% glycerol cryoprotectant and were frozen. The frozen precultures were thawed as needed. The bacteria were grown in brain heart infusion for 24 h at 37°C.

Apple cider was purchased from a local store, taking care that sorbic acid was not added, since this substance is very absorbent of UV light *(13)*. The cider was inoculated from the stationary-phase culture to give an approximately 6 log CFU/ml population.

The UV reactor consisted of a low-pressure mercury lamp assembly, a small-diameter coil of Chemfluor tubing surrounding the lamp, and a small-diameter coil of copper tubing surrounding the Chemfluor tubing. The UV lamp assembly (model EW-97505-00, Cole-Parmer, Vernon Hills, Ill.) contained a 15-W bulb (model T-15.C) that generated 90% of its energy at a wavelength of 254 nm, which inactivates bacteria, molds, yeast, and viruses *(16)*. The cylindrical bulb was 43.7 cm in length and had a diameter of 2.5 cm. Norton Chemfluor 367 tubing with an inside diameter of 1.6 mm and a wall thickness of 0.79 mm was wrapped around the entire length of the UV lamp. The length of the tubing in contact with the lamp was 1.1 m. The tubing has a UV transmission at 254 nm of 89%. Copper tubing with dimensions identical to those of the Chemfluor tubing was wrapped around the Chemfluor tubing. Finally, silicone tape was wrapped around the copper tubing to hold everything in place. Electrical tape was originally used, but it slowly deteriorated due to UV damage.

The experimental system included a feed tank and a peristaltic pump (driver model 7523-40, head model 77200-62, Masterflex tubing 96410-15, Cole-Parmer). The apple cider was pumped once through a stainless steel coil of 100-cm length that was submerged in a 25°C water bath (model Isotemp 1016S, Fisher Scientific, Pittsburgh, Pa.) and then through the UV processing Chemfluor tubing. The feed rate was varied from 27 to 83 ml/min, which was the maximum attainable with the pump-tubing combination. These rates translate into average treatment times of 19 to 58 s. The UV processing temperature was maintained by pumping 25°C water through the copper tubing that surrounded the processing tubing. Passage of cider through the UV reactor theoretically would have resulted in a temperature increase, depending on the flow rate, of 2.6 to 8.0°C, given the assumptions that all of the UV bulb's energy was transferred to the cider and that none of the heat was removed by the copper tubing. However, the copper tubing did remove heat and maintained the cider temperature at 25°C, as verified by measuring the temperature of the cider immediately after it exited the UV reactor using a 3.2-mm-diameter chrome-constantan thermocouple (Omega Engineering, Inc., Stamford, Conn.).

Product samples were collected in polypropylene tubes and placed on ice in a dark location to prevent photoreactivation *(7)*. Controls were performed by pumping the cider through the system while the UV lamp was off. Each experiment was performed in duplicate. Appropriate dilutions of the samples were plated on tryptose agar using a spiral plater (model DU2, Spiral Biotech, Bethesda, Md.) and incubated at 37°C for 24 h. Enumerations were made with a colony counter (model 500A, Spiral Biotech). Results were expressed as the means of these values ± the standard deviations.

RESULTS AND DISCUSSION

Both *E. coli* K-12 substrain C600 and *L. innocua* SA3-VT in apple cider were successfully inactivated using non-thermal UV irradiation. The extent of microbial inactivation was dependent on the bacterium and treatment time (or energy).

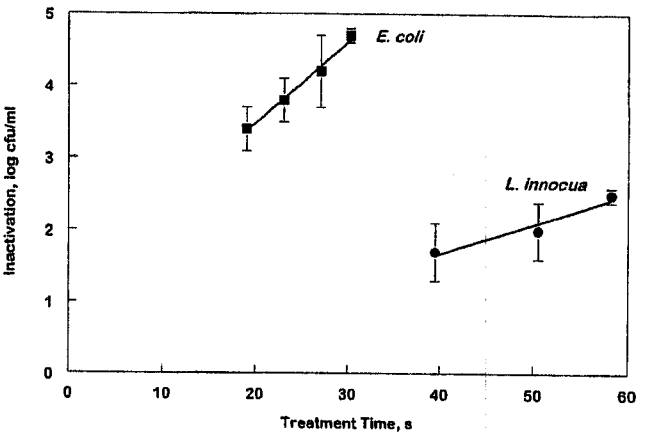

FIGURE 1. *Inactivation of* E. coli *and* L. innocua *as a function of treatment time at a processing temperature of 25°C.* ■, E. coli; ●, L. innocua. *Error bars indicate standard deviations.*

The best results were achieved with *E. coli.* The population of *E. coli* was reduced by 3.4 ± 0.3 log after being exposed for 19 s at a treatment temperature of 25°C. Lengthening the average treatment time to 30 s reduced *E. coli* to the limit of detection, equivalent to an inactivation of 4.7 log. Inactivations of approximately 5 log or greater have also been achieved using annular-type UV apparatuses *(4, 28)* and the California Day-Fresh Foods tubular UV apparatus *(19)*. The reduction was nearly linear ($r^2 = 0.99$) with respect to treatment time for the range of times investigated (Fig. 1). There was no sign of tailing, and the inactivation can be thought to follow first-order kinetics.

L. innocua was less sensitive to the UV treatment. Even at an average treatment time of 58 s, the reduction was only 2.5 ± 0.1 log. The ratio of the slopes of the linear regressions for the two bacteria indicated that *E. coli* was 2.7 times as sensitive as *L. innocua* to UV irradiation. Most of the reports in the literature on the UV processing of apple cider studied only *E. coli,* and none, to our knowledge, included *Listeria.* Kim et al. *(15)* investigated the effects of intensity and processing time of 254-nm UV irradiation on *Listeria monocytogenes* and *E. coli* inoculated into peptone water and onto stainless steel chips *(15)*. In the case of peptone water, after 1 min of UV treatment, *E. coli* and *L. monocytogenes* were reduced by 3.2 and 2.3 log, respectively. Similarly for the stainless steel case, *E. coli* and *L. monocytogenes* were reduced by 4.4 and 2.4 log, respectively. Shama *(23)* has reported that the UV dose required to reduce *L. monocytogenes* is 33% greater than that to reduce *E. coli.* However, Ramsay et al. *(21)* claimed that, in water, *E. coli* was slightly less sensitive than *L. innocua* to 254-nm UV irradiation *(21)*. *E. coli* and *L. innocua* were reduced by 2.2 and 2.9 log, respectively.

The energy cost of the UV processing of apple cider was determined. For a 58-s average treatment at a flow rate of 26.8 ml/min, the populations of *L. innocua* and *E. coli* were reduced by 2.5 log and greater than 4.7 log, respectively. On the basis of the flow rate and the UV lamp watt-

age, the energy applied was 34 J/ml, as determined by the following equation:

$$\text{Energy density} = \frac{\text{power}}{\text{flow rate}}$$

$$= \frac{15\ \text{W}}{26.8\ \text{ml/min}} \times \frac{1\ \text{J/s}}{1\ \text{W}} \times \frac{60\ \text{s}}{\text{min}} = 34\ \text{J/ml}$$

The energy cost for the UV process was $0.00046/liter of apple cider using the U.S. Department of Energy's data for the average industrial electric price for 2003 of $0.0495/kWh. For comparison, the energy costs for conventional thermal pasteurization, with heat regeneration or recovery, is approximately the same. Furthermore, the required energy for the nonthermal processes using pulsed or radio frequency electric fields is estimated to be in the range of 100 to 400 J/ml *(3, 10, 11, 22)*.

Additional studies are recommended. The UV process should be further scaled up to be of commercial interest. Norton Chemfluor 367 tubing with a larger inside diameter than 1.6 mm is available. The current flow rate of 83 ml/min could be increased to 400 ml/min or more. In addition, low-pressure mercury lamp assemblies with lengths greater than 44 cm and power ratings greater than 0.34 W/cm are available. The effect of installing several UV assemblies in series and applying several consecutive treatments to the cider should also be considered. Quality issues require investigation. Finally, the extension of the UV process to other liquid foods such as orange juice, vegetable juices, milk, egg, beer, and wine should be examined.

In conclusion, a straightforward UV apparatus was developed and assembled that inactivated bacteria in apple cider. It consisted of a low-pressure mercury lamp assembly surrounded by a small-diameter coil of Chemfluor tubing. The UV apparatus successfully inactivated both gram-positive and gram-negative bacteria at room temperature and at cider flow rates up to 83 ml/min. The population of *E. coli* was reduced by 3.4 ± 0.3 log after being exposed for 19 s. *L. innocua* was less sensitive to the UV treatment. Its population was reduced by 2.5 ± 0.1 log after being exposed for 58 s. The energy to UV process apple cider with a 58-s average treatment time was 34 J/ml. This is equivalent to an energy cost of $0.00046/liter and is comparable to the energy cost of thermal pasteurization. UV processing has the potential to improve the safety and extend the shelf life of apple cider while maintaining nearly all of its fresh-like qualities.

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